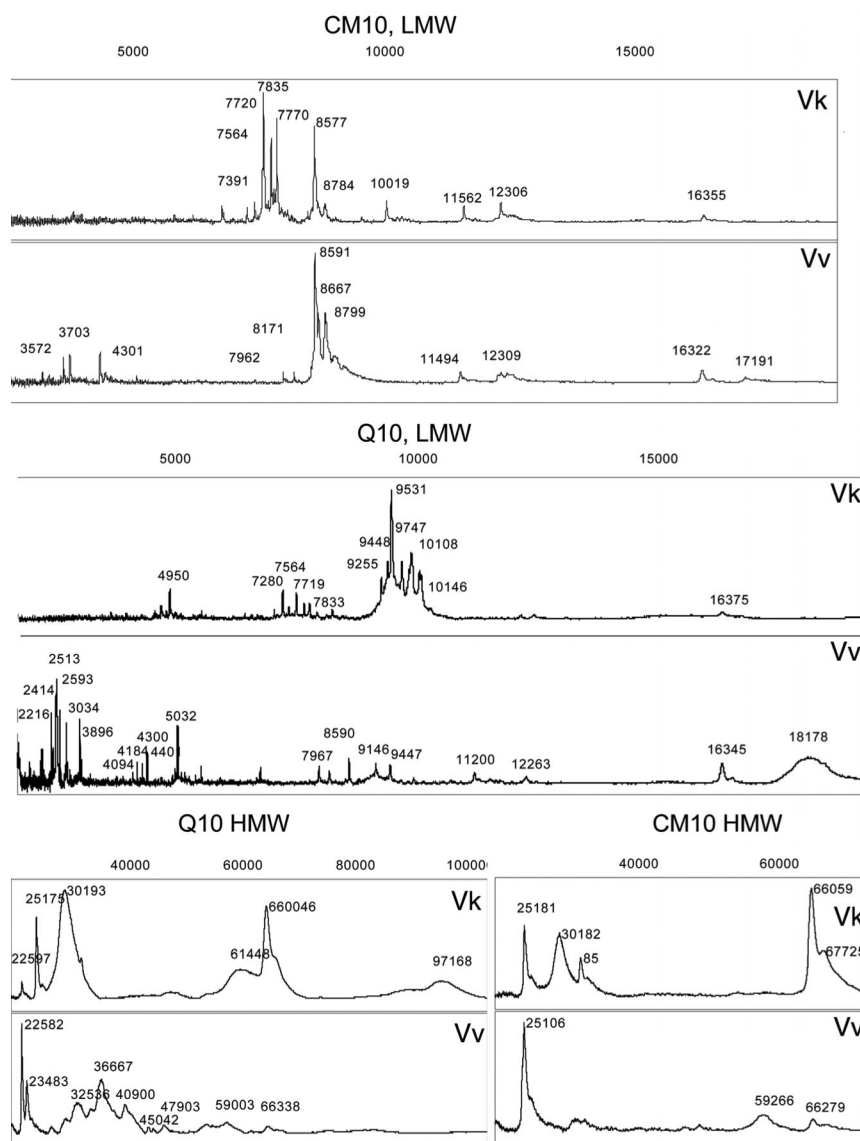
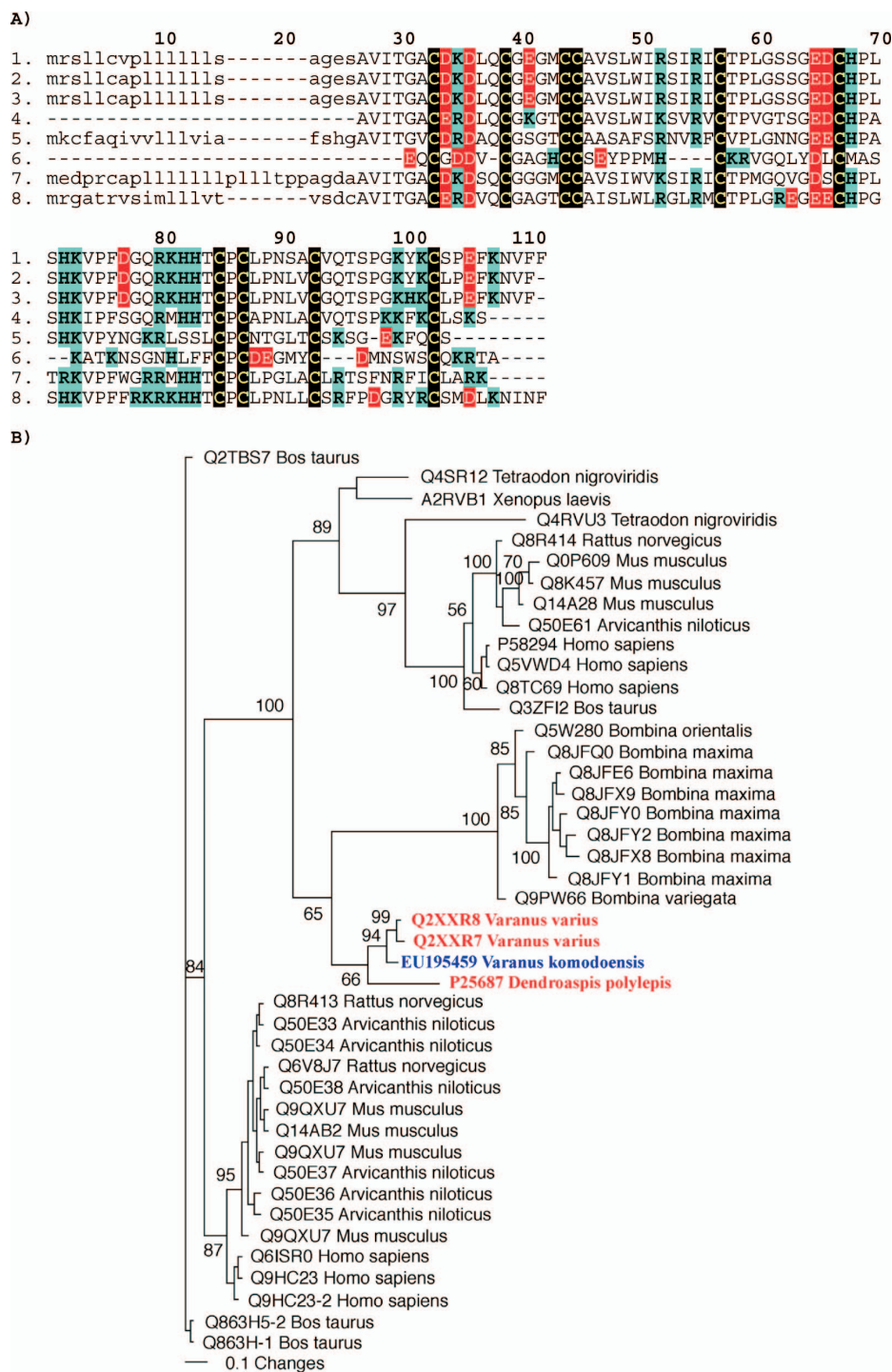


# Supporting Information

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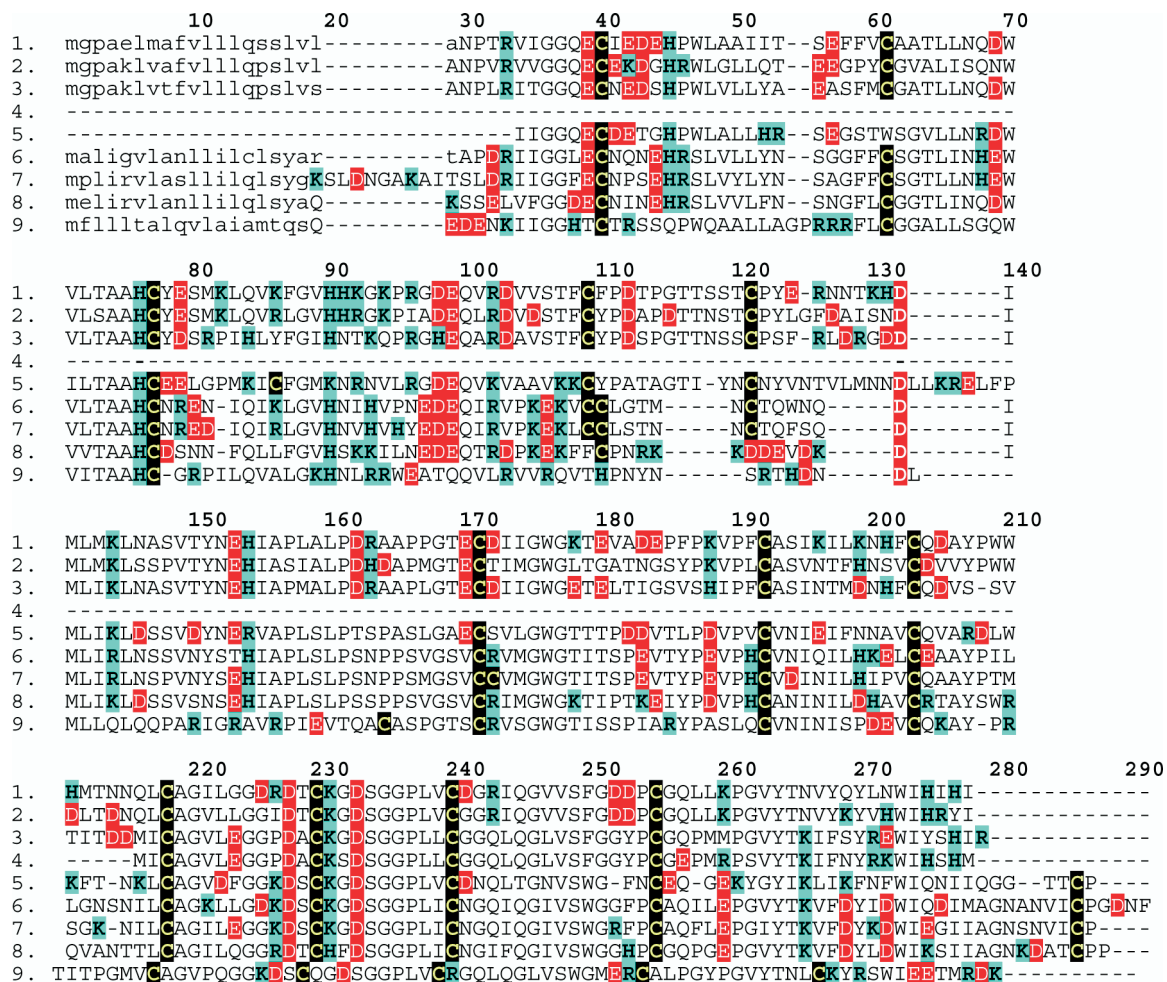


**Fig. S1.** SELDI-TOF MS comparison of *Varanus komodoensis* (Komodo Dragon) and *V. varius* (Lace Monitor) venoms using different arrays and wash buffers: Q10 (100 mM Tris-HCl, pH 9) and CM10 (20 mM sodium acetate, pH 5) in both the low (<20 kDa) and high mass ranges (>20 kDa).



**Fig. S2.** Molecular evolution of *Varanus komodoensis* AVIT toxins. (A) Sequence comparison of representative AVIT peptides: the reptile venom peptides (1) EU195459 from *Varanus komodoensis*, (2) Q2XXR8 and (3) Q2XXR7 from *V. varius*, and (4) P25687 from *Dendroaspis polylepis*; the amphibian toxic skin peptide (5) Q8JFY1 from *Bombina maxima*; the spider venom peptide (6) P81803 from *Hadronyche versuta*; and the nontoxin intestinal peptides (7) Q8R413 from *Rattus rattus* and (8) P58294 from *Homo sapiens*. Cysteines are highlighted in black with yellow font, and charged residues are shown in blue (positive) and red (negative). Signal sequences are shown in lowercase type. (B) Bayesian consensus tree. The outgroup is the nontoxin peptide Q2TBS7.





**Fig. S4.** Sequence comparison of representative kallikrein proteins: the reptile venom proteins (1) EU195456 and (2) EU195457 from *Varanus komodoensis*, (3) Q2XXN0 from *V. mitchelli*, (4) Q2XXN1 from *V. acanthurus*, (5) P43685 from *Heloderma horridum*, (6) Q09GK1 from *Philodryas olfersii*, (7) Q5MCS0 from *Lapemis curtus*, and (8) Q91516 from *Viridovipera stejnegeri*; and the nontoxin protein (9) Q9PUG3 from *Homo sapiens* shown for comparative purposes. Cysteines are highlighted in black with yellow font, and charged residues are shown in blue (positive) and red (negative). Signal sequences are shown in lowercase.

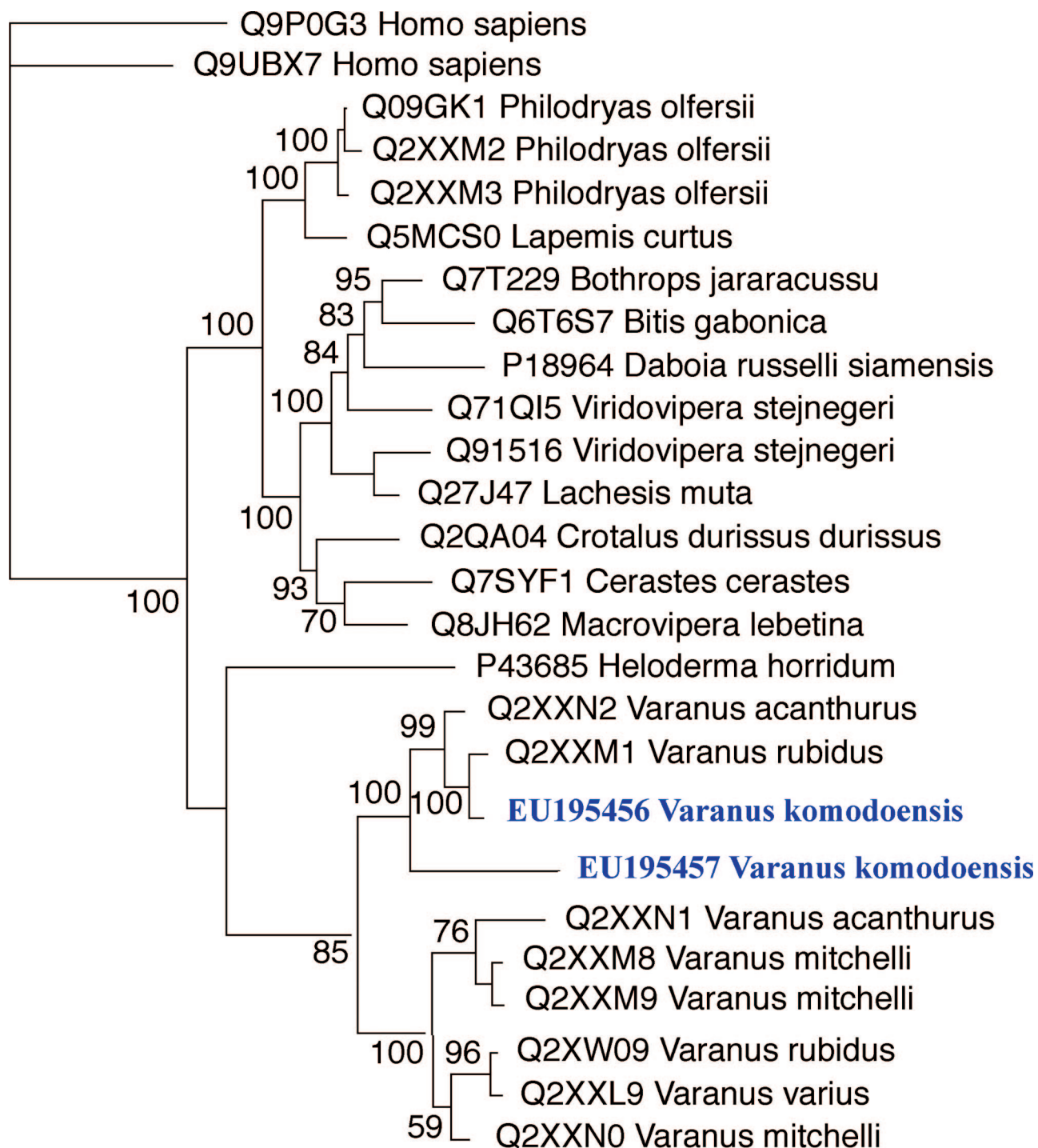


Fig. S5. Bayesian consensus tree of reptile kallikrein venom proteins. The outgroup is the nontoxin protein Q9P0G3.

10 20 30 40 50 60 70 80

1. SCCR<sup>E</sup>HDQCSVQITALQ<sup>R</sup>KHGIFNLR<sup>P</sup>YTISE<sup>C</sup>DCD<sup>T</sup>RF<sup>R</sup>TCLM<sup>D</sup>LN<sup>D</sup>TIA<sup>D</sup>FIGTTYFSVLQIP<sup>C</sup>FYLE<sup>E</sup>ES<sup>D</sup>EAC<sup>L</sup>EWA

2. SCCQ<sup>H</sup>HDQCSVQITALQ<sup>R</sup>KHGIFNLR<sup>P</sup>YTISE<sup>C</sup>DCD<sup>T</sup>RF<sup>R</sup>TCLM<sup>D</sup>LN<sup>D</sup>TIA<sup>D</sup>FIGTTYFSVLQIP<sup>C</sup>FYLE<sup>E</sup>ES<sup>D</sup>EAC<sup>L</sup>EW<sup>S</sup>

3. MCC<sup>R</sup>D<sup>H</sup>HCENWISAL<sup>E</sup>YKHGM<sup>R</sup>NYY<sup>P</sup>STISE<sup>C</sup>DCD<sup>N</sup>QFR<sup>S</sup>CLMK<sup>L</sup>KDGTAD<sup>Y</sup>VGQTYFNV<sup>L</sup>KIP<sup>C</sup>F<sup>E</sup>LEEG<sup>-</sup>EGCVD<sup>N</sup>N

4. MCC<sup>R</sup>D<sup>H</sup>HCSTMAAL<sup>E</sup>YKHGM<sup>R</sup>NYR<sup>P</sup>HTVSE<sup>C</sup>DCD<sup>N</sup>QFR<sup>S</sup>CLMN<sup>V</sup>KDRTAD<sup>L</sup>VGMTYFTVL<sup>K</sup>ISC<sup>F</sup>ELEEG<sup>-</sup>EGCVD<sup>N</sup>N

90 100 110 120 130 140 150

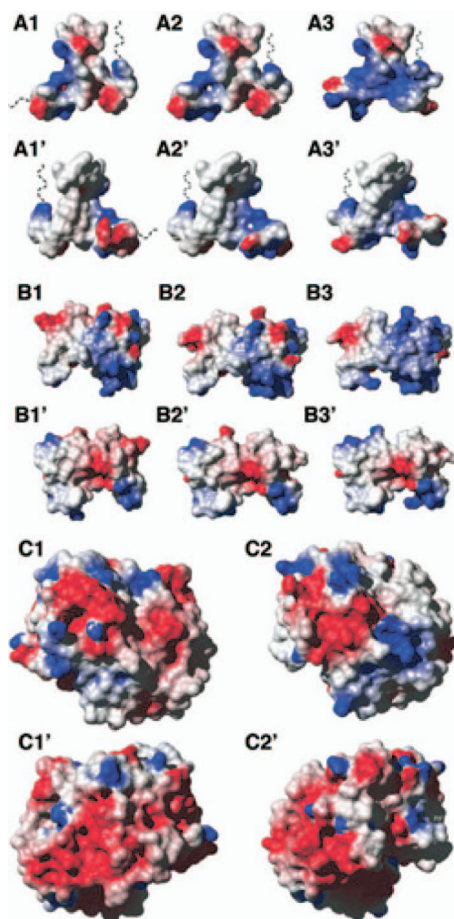
1. WWGG<sup>C</sup>NK<sup>R</sup>GRMLMA<sup>H</sup>TVPPSAYGSVPSVTTL<sup>P</sup>QPGT<sup>E</sup>TTK<sup>D</sup>R<sup>K</sup>HR<sup>K</sup>RKLQGP<sup>E</sup>HPGV<sup>P</sup>WQR<sup>R</sup>RP<sup>G</sup>SR<sup>R</sup>KL<sup>L</sup>L

2. W-----

3. FWL<sup>E</sup>CT<sup>E</sup>SKIMPVAKLVSAAPYQAQA<sup>E</sup>TQSG<sup>E</sup>GR-----

4. FSQQ<sup>C</sup>TKS<sup>E</sup>IMPVAKLVSAAPYQAQA<sup>E</sup>TQSG<sup>E</sup>EG-----

**Fig. S6.** Sequence comparison of representative lizard venom PLA<sub>2</sub> (type III) toxins: (1) EU195460 from *Varanus komodoensis*, (2) Q2XXL5 from *V. varius*, and (3) P16354 and (4) P80003 from *Heloderma suspectum*. Cysteines are highlighted in black with yellow font, and charged residues are shown in blue (positive) and red (negative). Signal peptides are not shown.



**Fig. S7.** Molecular modeling of representative reptile venom proteins. Blue surface areas indicate positive charges, red areas show negative charges, and model pairs show 2 sides of the protein rotated by 180°. Natriuretic peptides: (A1) EU195461 from *Varanus komodoensis* (Komodo Dragon), (A2) Q2XXL8 from *V. varius* (Lace Monitor Lizard), and (A3) P28374 from *Dendroaspis angusticeps* (Eastern Green Mamba). AVIT toxins: (B1) EU195459 from *V. komodoensis*, (B2) Q2XXR8 from *V. varius*, and (B3) P25687 from *D. polylepis* (Black Mamba). Kallikrein toxins: (C1) EU195456 from *V. komodoensis* and (C2) Q9I8X1 from *Deinagkistrondon acutus* (Sharp-nosed Pitviper).



**Movie S1:** Longitudinal MRI scans of *Varanus komodoensis* venom gland, proceeding from the outside of the gland inwards towards the teeth.

[Movie S1](#)



**Movie S2:** Transverse MRI scans of *Varanus komodoensis* venom gland, proceeding from posterior to anterior.

[Movie S2](#)



**Movie S3:** Longitudinal MRI scans of *Varanus komodoensis* venom gland, proceeding from ventral to dorsal.

[Movie S3](#)

Table S1. Muscle forces

Species	Muscle	Pretension, N	No. of trusses	Total, N
<i>V. komodoensis</i>	MAEM-a	0.935	10	9.35
	MAEM-(1)	0.663	4	2.65
	MAEM-(2)	0.682	6	4.092
	MAEM-b(3)	0.705	6	4.23
	MAEM-b(4)	0.824	10	8.24
	MPST-S	0.755	14	10.57
	MPST-P	0.931	4	3.72
	MAMP	0.953	6	5.72
	MPT	1.800	36	64.80
	MAEM-d	1.034	12	12.41
	MAEM-c	0.714	12	8.57
	MAEM-S	12.872	12	154.46
<i>C. porosus</i>	MAEM-P	13.645	10	136.45
	MAEM-M	6.770	12	81.24
	MAMP	12.905	34	438.77
	MPT-P	6.933	68	471.44
	MPT-A	4.310	34	146.54
	MPST	7.785	10	77.85
	MIM	11.005	10	110.05

Heterogeneous tet4 finite element models of *Crocodylus porosus* (AM5779) and *Varanus komodoensis* (AM R106933) were assembled on the basis of serial x-ray (CT) data acquired in DICOM format by using a Toshiba Aquilion 16 scanner (Mater Hospital, Newcastle, New South Wales, Australia). Models comprised 1,613,150 and 1,293,458 "brick" elements, respectively. Forces and truss numbers for individual muscles are shown. MAEM (a–d), m. adductor externus mandibulae; MAEME, MAE-M eternus; MAEM-P, MAEM profundus; MAEM-M, MAEM medialis; MPST, m. pseudotemporalis; MPST-S, MPST superficialis; MPST-P, MPST profundus; MAMP, m. adductor mandibulae posterior; MPT, m. pterygoidus; MPT-A, MPT anterior; MPT-P, MPT posterior; MIM, m. intramandibularis. N, newtons. Jaw adductor forces for each muscle were based on estimates of cross-sectional areas (X-Phys). These were calculated on the basis of muscle volumes, with volumes divided by truss lengths to provide an X-Phys for each muscle: truss diameter (mm) =  $2 \times ((X\text{-Phys})/\pi)^{0.5}$ . Trusses were arranged in proportion to muscle masses/volumes, with truss volumes calibrated to fit with initial values. Muscle forces were predicted from this calibration.